Etiology of sepsis in Uganda using a quantitative PCR-based TaqMan Array Card

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Summary: Using a qPCR TaqMan Array Card, we found that cytomegalovirus and *Mycobacterium tuberculosis* were the most frequently detected targets in the blood of patients with sepsis in Uganda and both were independently associated with increased mortality.

Running title: Etiology of sepsis in Uganda

Abstract

Background. Knowledge of causes of sepsis in sub-Saharan Africa is limited. A better understanding of the microbiology of bloodstream infections could improve outcomes.

Methods. We used a quantitative PCR (qPCR)-based TaqMan Array Card (TAC) to directly test for 43 targets from whole blood. We analyzed 336 cryopreserved specimens from adult Ugandans with sepsis enrolled in a multi-site study; 84% were HIV-infected. We compared qPCR TAC results with blood culture and determined the association of qPCR with study participant outcomes using logistic regression.

Results. The most frequently detected targets were cytomegalovirus (CMV, n=139, 41%), *Mycobacterium tuberculosis* (TB, n=70, 21%), *Plasmodium* (n=35, 10%), and *Streptococcus pneumoniae* (n=31, 9%). Diagnostic performance varied by target with qPCR sensitivity averaging 61±28% and specificity 98±3% versus culture. In multivariable analysis, independent factors associated with in-

hospital mortality included CMV viremia (adjusted odds ratio [aOR] 3.2, 95% CI, 1.8-5.5; p<.01) and TB qPCR-positivity, whether blood culture-positive (aOR 4.6, 95% CI, 2.1-10.0; p<.01) or blood culture-negative (aOR 2.9, 95% CI, 1.2-6.9; p=.02).

Conclusions. Using qPCR TAC on direct blood specimens, CMV and TB were the most commonly identified targets and were independently associated with increased in-hospital mortality. qPCR TAC screening of blood for multiple targets may be useful to guide triage and treatment of sepsis in sub-Saharan Africa.

Key words: Sepsis; Africa; PCR; Uganda; Mortality

Introduction

Sepsis is a common cause of hospital admission, morbidity, and mortality in sub-Saharan Africa (1;2). In resource-limited settings such as much of sub-Saharan Africa, little is known about the epidemiology of sepsis. A better understanding of the microbiology of sepsis would help clinicians in the region to select appropriate antimicrobial therapy, avoid unnecessary therapy, and thereby improve outcomes. Unlike sepsis cohorts in North America and Europe, there is a high prevalence (60-90%) of HIV infection in patients with sepsis in sub-Saharan Africa which is likely to have an impact on the etiology of their infections (3-5).

The spectrum of possible bloodstream infections is broad, and many are not identified through standard culture methods (6). For example, there is a high rate of *Mycobacterium tuberculosis* (TB) bloodstream infections in HIV-infected patients (7;8), but mycobacterial blood cultures are rarely performed. Other

pathogens such as *Leptospira, Coxiella*, and *Brucella* are identified through serology which often requires initial and follow-up samples that are collected several weeks later (9-12). Viral testing is virtually never performed other than in specialized settings (5;13-15).

Given the high cost, low yield, and slow turnaround time of standard bacterial cultures, molecular techniques offer broad diagnostic potential. We recently described the development of a TaqMan Array Card (TAC) for the investigation of acute febrile illnesses using blood samples (16). This technology allows for simultaneous, single-plexed, quantitative PCR-based amplification of multiple targets including bacteria, viruses, fungi, and protozoa with sensitivity and specificity of 88% and 99%, respectively, compared to the cognate assays (16). Here we used the qPCR TAC to determine the underlying cause of infection in patients enrolled in a prior study of fluid resuscitation in septic patients in Uganda (17). We compared the results of qPCR TAC with prior clinical microbiology testing and examined the association of qPCR TAC results with the important clinical outcome of in-hospital mortality.

Methods

Specimens. We obtained a convenience sample of available cryopreserved EDTA-whole blood specimens from 336 adult patients aged ≥18 years with sepsis enrolled in a fluid resuscitation study from the medical casualty units of Mulago National Referral Hospital and Masaka Regional Referral Hospital in Uganda from May 2008 to May 2009 (17). Written informed consent was obtained from each patient or a surrogate if the patient could not provide consent. At the time of enrollment, we obtained baseline demographic and clinical data including antibiotics received. We collected blood from eight

healthy Ugandan volunteers as negative controls. All whole blood samples were cryopreserved at -80°C until they were tested in 2015-2016.

Target detection using qPCR TAC. We incorporated primers and probes for 43 targets and two extrinsic controls on the TAC as previously described (Supplemental Tables 1 and 2) (16). Any quantification cycle (Cq) result of <40 was considered positive. Lower Cq values indicate higher quantity of target material present in the sample.

Cryptococcal antigen testing. We tested a convenience sample of available plasma specimens from 265 participants for the presence of cryptococcal antigen using lateral flow assay kits (Immuno-Mycologics Inc., Norman, OK, USA) following the manufacturer's instructions.

Statistical analysis. We determined sensitivity and specificity for qPCR TAC results compared to standard culture results. We summarized participant characteristics as percentage for categorical variables and median with interquartile range (IQR) for continuous variables. We compared proportions with the Chi-square test and compared continuous variables with the Mann-Whitney U test. We evaluated relationships between qPCR TAC results and in-hospital mortality with logistic regression and adjusted for age, sex, and HIV infection. P values were two-tailed, and <.05 was considered significant (SPSS, Version 22.0; IBM Corp, Armonk, New York).

Results

Demographics and qPCR TAC results. Of the 336 participants, the median (IQR) age was 34 (27-40) years, 175 (52%) were female, 282 (84%) were HIV-infected, and 90 (27%) died in-hospital (Table 1). The clinical and laboratory data from the convenience sample were similar to those from the total enrolled cohort (Supplemental Table 3) (17). All 336 samples yielded valid qPCR TAC results as described in Supplemental Table 2. We tested positive samples again for the relevant targets with PCR assays that generated longer amplicons (16). As seen previously, ~80% (277 of 346) of the TAC results were sequence-confirmed, and those that were able to be confirmed by long amplicon sequencing had significantly lower Cq values than those that were not (31.1±4.7 vs 36.6±1.7, p<.01) (16).

No target was detected in any of the 8 Ugandan healthy volunteer controls. We detected at least one target in 245 of 336 participants (72%, Table 2). The most frequently detected targets were cytomegalovirus (CMV, n=139, 41%), TB (n=70, 21%), *Plasmodium* species (n=35, 10%), and *Streptococcus pneumoniae* (n=31, 9%). Excluding CMV, 138 (41%) participants had one target, 18 (5%) had two targets, and 1 (0.3%) had three targets identified by qPCR. Of the 139 cases of CMV viremia, there were 66 (40%) cases of bacterial coinfection including 28 (42%) that were identified by both qPCR and culture, 25 (38%) that were identified by qPCR only, and 13 (20%) that were identified by culture only. Compared to previously performed blood cultures, qPCR TAC performance varied by target averaging 61% sensitivity and 98% specificity (Table 3). Overall, culture-positive samples had lower Cqs (31.4±3.1, n=65) than those that were culture-negative (33.1±3.0, n=61, p<.01).

qPCR TAC results and in-hospital mortality. CMV viremia was associated with in-hospital mortality, with 57 of 138 (41%) CMV-positive participants dying in hospital compared to 33 of 195 (17%) CMV-negative participants (Odds Ratio 3.5, 95% confidence interval [CI] 2.1-5.7, p<.01). We did not have any pathology

data to determine end-organ involvement by CMV versus reactivation viremia in the setting of critical illness. There were 40 (12%) TB qPCR-positive/culture-positive cases, 30 (9%) TB qPCR-positive/culture-negative cases, 29 (9%) TB qPCR-negative/culture-positive cases, and 237 (71%) TB qPCR-negative/culture-negative cases. The in-hospital mortality for these groups was 55%, 43%, 31%, and 20%, respectively (Table 4). In multivariable analysis, CMV viremia (adjusted Odds Ratio 3.19, 95% CI 1.83-5.54, p<.01) and a positive TB qPCR result with either a concurrent negative (adjusted Odds Ratio 2.85, 95% CI 1.18-6.88, p=.02) or positive (adjusted Odds Ratio 4.60, 95% CI 2.12-9.98, p<.01) TB culture result were independently associated with in-hospital mortality (Table 4).

The in-hospital mortality for *S. pneumoniae* qPCR-positive cases (n=31) was 33% compared to 13% in-hospital mortality for qPCR-negative/culture-negative cases (adjusted Odds Ratio 2.18, 95% CI 0.89-5.30, p=.09). *Plasmodium* qPCR-positive cases had lower mortality (6%); however, there was not a statistically significant difference in mortality when compared to qPCR-negative/culture-negative cases (adjusted Odds Ratio 0.25, 95% CI 0.06-1.16, p=.08). Of the two *Plasmodium* infected participants that died, one had CMV viremia, and the other had *S. pneumoniae* coinfection.

Seventeen of 265 participants (86% HIV-infected) had positive cryptococcal antigen (CrAg) assays; 6 were also *Cryptococcus* qPCR-positive and 5 were *Cryptococcus* blood culture-positive. All positive CrAg results occurred in HIV-infected participants. In-hospital mortality for these participants was 59% (CrAg), 83% (*Cryptococcus* TAC), and 60% (*Cryptococcus* blood culture). The sensitivity of qPCR for identifying *Cryptococcus* versus culture was 80%. The sensitivity of qPCR for identifying CrAg assay positive cases was 29% and specificity was 100%. The qPCR sensitivity improved to 55% (6 of 11) for cases with CrAg

assay titer ≥1:2560. Four of 5 cases with low CrAg assay titers (<1:80) had a second causative pathogen identified in blood culture.

Quantity of amplified DNA and in-hospital mortality. The quantities of TB, CMV, and *S. pneumoniae* DNA as measured by qPCR (Cq) appeared to directly correlate with mortality (Figure 1). In multivariable analysis that included continuous Cq values for CMV and TB, we found an adjusted Odds Ratio of in-hospital mortality of 1.4 (95% CI 1.2-1.7, p<.01) and 1.6 (95% CI 1.2-1.9, p<.01) for each ten-fold increase in quantity for CMV and TB, respectively.

Antimicrobial therapy and in-hospital mortality. There were 43 different combinations of antibacterial therapy administered to 324 of 336 (96%) participants in our study. The most frequently administered antibacterial regimen was ceftriaxone monotherapy which was administered to 77 (23%) participants. Antifungal therapy was administered to 25 (9%) participants and anti-mycobacterial therapy was administered to 32 (11%) participants. No study participants received anti-viral therapy other than anti-retroviral therapy. There were 120 participants with positive blood cultures. Of the 100 participants with positive blood cultures and known antibiotics, 39 (39%) received appropriate antibiotics and of the 45 participants with negative blood cultures and positive TAC results, 29 (64%) received appropriate antibiotics.

Mortality from TB infection remained high despite anti-TB therapy. There were 13 (13.1%) of 99 confirmed TB cases (by culture, qPCR, or both) who received empirical anti-TB therapy, 4 (31%) of whom

died in-hospital. This mortality contrasts with 48% in-hospital mortality among TB cases who did not receive anti-TB therapy but the difference is not statistically significant (Odds Ratio 2.07, 95% CI 0.60-7.34, p=.25). Excluding TB, 53 of 71 (75%) patients with a positive qPCR result for a bacterial infection that received appropriate antibiotics had an in-hospital mortality of 23% compared with 33% in those who did not receive appropriate antibiotics (Odds Ratio 0.59, 95% CI 0.18-1.89, p=0.37).

Discussion

In this work, we demonstrate the utility of a multi-target qPCR TAC in the evaluation of sepsis in sub-Saharan Africa. We found CMV and TB were the most commonly identified TAC targets in the blood of patients with sepsis in Uganda and were independently associated with increased in-hospital mortality. Future use of qPCR TAC to screen blood for multiple targets may be useful for therapeutic triage of individual patients, outbreak investigations, and determination of local epidemiology through use in sentinel laboratories.

There are abundant data that indicate that the time to appropriate therapy for bloodstream infections correlates with improved mortality (18-20). However, given the large number of potential pathogens, there is a need for improved syndromic diagnostic methods. Blood culture, considered the gold standard for microbiological diagnosis, is expensive, requires several days to yield a result, and is affected by antibiotic pre-exposure which is common in many settings. Serologic tests are often unavailable. PCR tests are faster and can be designed for virtually any pathogen. Previous studies using commercial PCR-based methods to detect pathogens directly in

blood including SeptiFast®, SepsiTest™ and IRIDICA BAC BSI Assay used ≥2mL of blood and revealed only a 48-84% sensitivity compared with blood culture (21). This low to moderate sensitivity range presumably reflects low numbers of pathogens in blood, as has been clearly demonstrated for *Salmonella enterica* Typhi (22).

Other challenges to PCR in blood are the typically low sample volumes and inhibitory substances within blood. In this context, we were not surprised to observe a 61% overall sensitivity of qPCR TAC versus culture, particularly given the sub-microliter volumes of nucleic acid required for qPCR TAC. We were limited to less than 2mL of whole blood per sample in this study and we would advocate using larger volumes to improve sensitivity. Another factor was that these blood samples were stored ~7 years prior to testing, thus specimen degradation could have occurred. However, it has been shown that DNA in blood samples frozen at -80^{o}C for 16 months was preserved well (23).

A systematic review of commercial PCR methods used to identify bacteria and fungi in blood found 81-86% specificity versus blood culture, in other words a 14-19% “false positive” rate (21). An important finding of ours is that the qPCR-positive/culture-negative specimens were not false information but were clinically significant. Specifically, a TB qPCR-positive case, even if culture-negative, exhibited a high mortality. In fact, the TB qPCR-positive/culture-negative mortality was higher than and maintained statistically significant prognostic information over the TB qPCR-negative/culture-positive mortality. This finding combined with the inverse relationship between Cq values and mortality suggests a higher burden of infection for TB qPCR-positive/culture-negative cases compared to TB qPCR-negative/culture-

positive cases. Therefore we feel qPCR-positive/culture-negative results in blood should be re-examined and not reflexively dismissed.

As for the TB qPCR TAC performance itself versus culture, our results are similar to most other studies. A study found a sensitivity of 42% and specificity of 100% for PCR compared to a gold standard of TB culture positivity from any clinical specimen (24). A small study from Malawi reported a 56% concordance between Xpert MTB/RIF PCR detection from blood and blood culture in HIV-infected patients with a high clinical suspicion for TB (25). In a study from Uganda, PCR identified TB in blood with a sensitivity and specificity of 33% and 97% respectively when using 2 mL samples and 71% and 96% respectively when using 10 mL samples (26).

The 50% TB mortality we observed was also expected. This finding is similar to studies from Malawi, South Africa, Tanzania, and Uganda which found an associated mortality of 21-50% (7;27-29). In our study, few patients received anti-TB therapy. A trend towards lower mortality was observed in TB patients who received anti-TB therapy versus those who did not, although mortality remained high and the difference was not statistically significant. In 317 HIV-infected sputum smear-negative patients presenting with clinical danger signs in Uganda, mortality was 20% if patients received empiric anti-TB therapy compared to 36% mortality if they did not (30). In another study of 34 patients in Uganda with confirmed mycobacteremia, the proportion of patients who died at one year was significantly lower in those who were receiving anti-TB therapy (23%) at the time of culture positivity compared with those who were not receiving anti-TB therapy (58%) (28). These results suggest that early identification and

treatment of mycobacteremia can improve outcomes, and TB should be raised on the differential diagnosis of inpatients with sepsis in TB endemic settings.

CMV was the most frequent qPCR TAC target found in this patient population and CMV viremia was independently associated with mortality. In critically ill patients, CMV reactivation is known to be associated with increased mortality; however, CMV viremia does not usually require treatment (31-34). A randomized clinical trial of ganciclovir for critically ill CMV-seropositive adults showed no difference in mortality between treatment groups (34). CMV seroprevalence is approximately 80% in sub-Saharan Africa, and CMV viremia in dried blood spots was independently associated with increased risk of death in HIV-infected patients from Tanzania (35-37). Although we did not have clinical data regarding end-organ damage due to CMV viremia, given the high number of bacterial coinfections in CMV viremia cases, we believe the CMV viremia was likely a prognostic marker but not causal for mortality. In support of the association between CMV reactivation and underlying disease severity is our finding that lower Cq values were associated with increased mortality in our multivariable analysis.

The three leading qPCR TAC targets we detected, CMV, TB and *S. pneumoniae*, all exhibited an inverse relationship between Cq and in-hospital mortality. Therefore, PCR positivity may reflect a higher burden of infection, which is associated with mortality. Overall *S. pneumoniae* was associated with considerably lower mortality than TB perhaps because standard therapy for critically ill patients would be likely to treat this pathogen. However *S. pneumoniae* showed a high mortality similar to TB at its highest bacterial load (i.e., lowest Cq), perhaps because of overwhelming infection. We presume the low

mortality associated with *Plasmodium* positivity was because this may have represented incidental asymptomatic parasitemia (38).

There were also some cases of *Cryptococcus*, Dengue virus, and *Rickettsia* species identified by qPCR TAC. While *Cryptococcus* can be readily identified by the antigen test, cryptococcosis remains associated with high mortality rates (39). Since 4 of 5 cases with low CrAg assay titers (<1:80) had a second causative pathogen identified in blood culture, it is unlikely that cryptococcosis was the etiology of sepsis in these cases. Dengue and *Rickettsia* are not easily recognized or considered as pathogens in critically ill patients in this region. However, spotted fever and typhus group rickettsiosis have been identified in febrile patients in Tanzania and *Rickettsia* species are commonly found in dog fleas in Uganda (12;40). Rickettsial infections are treatable with doxycycline and a better understanding of which patients are likely to present with a rickettsial infection could help guide empiric therapy.

There were limitations to this study. While the overall sample size of 336 was substantial, on a per pathogen basis we did not have sufficient power to conduct all analyses. Although we tested for 43 pathogens, there are still others that one might want to include. Not all qPCR TAC targets could be confirmed through standard testing due to specimen availability. With regard to CMV specifically, detection of CMV in whole blood is generally greater than that obtained in plasma, and plasma has been advocated as a better sample type for measuring CMV response to therapy, however that was not our goal or of great relevance to this setting (41;42). Additionally, the qPCR TAC platform is well-suited to high throughput testing but less well-suited to clinical care because it requires a manual nucleic acid

extraction method and tests 8 samples at once. Although molecular testing is expensive (~$50-100 reagent costs per sample), blood culture and other methodologies are also costly and imperfect.

In summary, in a predominantly HIV-infected population with sepsis, we confirmed that TB is the leading cause of bacteremia which is associated with high mortality. CMV viremia was also independently associated with mortality. In total we identified 19 different targets including many that would not be treated with standard empiric antibiotics (e.g. *Rickettsia* species) or require treatment (e.g. Dengue virus). We believe this work demonstrates the principle that a pan-molecular quantitative assay that screens for multiple targets in blood would be useful to provide therapeutic and prognostic information, particularly in settings with a high burden of HIV and other infectious diseases. For example, the qPCR TAC assay could identify the need for early TB therapy, CMV viremia as a marker for early mortality, and *S. pneumoniae* as an indicator of intermediate mortality (33%); or if nothing is positive with a large panel, this could portend a better prognosis.

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Conflict of interest

The authors report no conflicts of interest

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Figure legend

Figure 1. Association of Cytomegalovirus (CMV), *M. tuberculosis*, *Plasmodium, S. pneumoniae*, and DNA quantity as determined by quantification cycle with mortality for 336 adult patients admitted to hospital with sepsis in Uganda. The x-axis shows the Cq ranges, while the y-axis shows the in-hospital mortality associated with each pathogen according to the corresponding Cq cutoff. The dark bar indicates the mortality rate for TAC negative patients for all the pathogens interrogated.

Table 1. Clinical and laboratory features of 336 adult hospitalized patients with sepsis in Uganda.

Clinical or laboratory variable	*median (IQR) or n (%)*
Age, years	34 (27-40)
Women	175 (52%)
HIV-infected	282 (84%)
CD4+ T cells/μL	61 (13-176)
Lactate, mmol/L	3.9 (3.0-4.9)
In-hospital mortality	90 (27%)

Table 2. qPCR TAC detection in blood from 336 adult patients admitted to hospital with sepsis in Uganda.

Pathogen, n (%)	***Total (n=336)***	***HIV-infected* (n=282)***	***HIV-negative (n=50)***
CMV	139 (41)	129 (46)	8 (16)
Mycobacterium tuberculosis	70 (21)	63 (22)	6 (12)
Plasmodium species	35 (10)	18 (6)	17 (34)
Streptococcus pneumoniae	31 (9)	27 (10)	4 (8)
Dengue	17 (5)	16 (6)	1 (2)
Escherichia coli	16 (5)	11 (4)	5 (10)
Cryptococcus neoformans	6 (2)	6 (2)	0 (0)
Rickettsia species	6 (2)	4 (1)	2 (4)
Pseudomonas aeruginosa	4 (1)	2 (0.7)	2 (4)
Klebsiella pneumoniae	4 (1)	4 (1)	0 (0)
Staphylococcus aureus	4 (1)	3 (1)	1 (2)
Toxoplasma gondii	4 (1)	3 (1)	1 (2)
Acinetobacter baumannii	3 (0.9)	3 (1)	0 (0)
Salmonella species	3 (0.9)	3 (1)	0 (0)
Hepatitis E	2 (0.6)	1 (0.4)	1 (2)
Leptospira species	2 (0.6)	2 (0.7)	0 (0)
Brucella species	1 (0.3)	1 (0.4)	0 (0)
Coxiella burnetii	1 (0.3)	1 (0.4)	0 (0)
Salmonella enterica Typhi	1 (0.3)	1 (0.4)	0 (0)

No specimens were positive for: *Bartonella* species, Crimean-Congo hemorrhagic fever, Chikungunya, Enterovirus, Group B *Streptococcus*, *Haemophilus influenzae*, Hantavirus, *Histoplasma capsulatum, Listeria monocytogenes, Leishmania* species*, Mycobacterium avium* complex, Marburg*, Neisseria meningitidis*, Nipah, O'nyong'nyong, Rift Valley Fever, Sudan*, Trypanosoma brucei*, West Nile*, Yersinia pestis*, Yellow Fever, or Zika.

*HIV serostatus was known for 332 (99%) of the 336 study participants

Table 3. Comparison between qPCR TAC and blood culture from 336 adult patients admitted to hospital with sepsis in Uganda. Average PCR Cycle of Quantification (Cq) and standard deviation are shown in parentheses.

Pathogens	***Culture positive***			***Culture negative***		
	qPCR +	qPCR -	sensitivity	qPCR +	qPCR -	specificity
M. tuberculosis	40 (31.2±3.4)	29	58%	30 (32.1±2.9)	237	89%
S. pneumoniae	16 (31.3±2.9)	4	80%	15 (32.8±3.2)	301	95%
Cryptococcus	4 (30.9±2.0)	1	80%	2 (31.3±4.6)	329	99%
Salmonella	2 (31.8±1.3)	2	50%	1 (38.2)	331	100%
S. aureus	1 (33.5)	3	25%	3 (33.1±2.2)	329	99%
E. coli	3 (34.8±0.6)	0	100%	13 (34.6±1.8)	320	96%
Pseudomonas	1 (33.1)	0	100%	4 (36.6±2.3)	331	99%
K. pneumoniae				4	332	99%
A. baumannii				3	333	99%

Total	65	41	61%	61	3193	98%

Table 4. Univariable and multivariable analysis of predictors of in-hospital mortality for 336 adult patients admitted to hospital with sepsis in Uganda

Characteristic	*Number (%)*	*Mortality, n (%)*	*Odds ratio*	*95% Confidence interval*	*P*	*Adjusted odds ratio*	*95% Confidence interval*	*P*
Age, per year	-	-	1.01	0.98-1.03	0.64	1.00	0.97-1.03	0.83
Women	175 (52)	41 (23)	0.68	0.42-1.11	0.12	0.86	0.49-1.51	0.60
HIV-infected	282 (84)	84 (30)	3.73	1.43-9.75	0.01	1.62	0.56-4.70	0.37
CMV qPCR+	139 (41)	57 (41)	3.46	2.09-5.72	<0.01	3.19	1.83-5.54	<0.01
qPCR and culture neg	68 (20)	9 (13)	1.0	-	-	1.0	-	-
TB qPCR neg and culture +	29 (9)	9 (31)	1.24	0.54-2.83	0.61	1.87	0.75-4.67	0.18
TB qPCR+ and culture neg	30 (9)	13 (43)	2.24	1.04-4.83	0.04	2.85	1.18-6.88	0.02
TB qPCR+ and culture +	40 (12)	22 (55)	4.04	2.05-7.98	<0.01	4.60	2.12-9.98	<0.01
S. pneumoniae qPCR+	31 (9)	10 (33)	1.39	0.63-3.11	0.41	2.18	0.89-5.30	0.09
Other bacteria qPCR+	42 (13)	9 (21)	0.71	0.32-1.54	0.38	0.73	0.30-1.78	0.49
Plasmodium qPCR+	35 (10)	2 (6)	0.15	0.03-0.62	0.01	0.25	0.06-1.16	0.08

+ = positive; neg = negative

Figure 1.